Chronic Cryptococcal Meningitis

A New Experimental Model in Rabbits

John R. Perfect, MD, Selwyn D. R. Lang, MB, FRACP,
and David T. Durack, DPhil, MD

This paper describes the salient features of a new model for chronic cryptococcal meningitis in cortisone-treated rabbits. Normal rabbits soon recovered after intracisternal inoculation of *Cryptococcus neoformans*, but cortisone-treated animals developed chronic progressive meningitis that was fatal in 2-12 weeks. Incidence and severity of infection was related to cortisone dose, not to inoculum size. The number of mononuclear cells that migrated into the subarachnoid spaces and cerebrospinal fluid of infected rabbits was strikingly reduced by cortisone treatment. Rabbits with cryptococcal meningitis were febrile; their high body temperature did not confer resistance to this infection. Cortisone-treated rabbits provide a new and expedient laboratory model for cryptococcal disease. Potential applications include study of the pathogenesis of cryptococcosis, investigation of the immunobiology of the CNS in chronic meningitis, and *in vivo* evaluation of newer anticryptococcal treatment regimens. (Am J Pathol 1980, 101:177-194)

CRYPTOCOCCUS NEOFORMANS is a low-grade pathogen that sometimes causes chronic meningitis or disseminated infection in man.[1-3] In these potentially fatal cases some form of underlying immune compromise is commonly present.[4,5] Progress in investigation of the relevant immune mechanisms has been limited by lack of a convenient animal model, simulating the disease in man and allowing repeated analysis of cerebrospinal fluid (CSF).

The rabbit, although a convenient animal for experimental purposes, has seldom been used because this species seems to be innately resistant to cryptococcal infection. Rabbits tolerated intraperitoneal, intravenous, and intracisternal injection of large inocula of *C neoformans* without developing lasting or fatal infection[1] (Shadomy J, personal communication). Localized infections have been produced in the anterior chamber of the eye[6] and in the skin [7] by direct inoculation of cryptococci, but dissemination did not occur. Felton et al [8] produced localized pulmonary lesions in rabbits by direct inoculation of *C neoformans* into the lungs. They observed a mononuclear inflammatory reaction in the meninges and noncaseating granulomas within the brain, but they could not demonstrate cryptococci in the central nervous system by microscopy or culture. One theory that has been offered to explain the natural resistance of rabbits to *C neo-*

From the Division of Infectious Diseases, Duke University Medical Center, Durham, North Carolina.

Accepted for publication May 14, 1980.

Address reprint requests to Dr. John R. Perfect, Box 3867, Duke University Medical Center, Durham, NC 27710.

0002-9440/80/1008-0177$01.00

formans is that their normal body temperature is high enough to inhibit the growth of this fungus *in vitro.*[1]

Corticosteroids reduce the natural resistance of humans and animals to infection. The magnitude of this effect, which varies between species, has been extensively studied in laboratory animals exposed to various potentially pathogenic organisms.[9] For example, Louria et al [10] described the effects of corticosteroids on murine infection caused by *Candida albicans*, *Histoplasma capsulatum*, and *C neoformans.* The incidence and severity of candida infection was potentiated, but they found no difference in mortality between control and cortisone-treated mice inoculated with histoplasma or cryptococci. Gadebusch and Gikas [11] compared the effects of cortisone on pulmonary cryptococcosis in the guinea pig and the rat. Cortisone treatment made the disease more severe in rats, and latent infection could be reactivated by cortisone in both species. Diamond [12] showed that cortisone depressed both immune and inflammatory responses and shortened survival in female guinea pigs infected with *C neoformans.* In rabbits, corticosteroids have been shown to enhance the virulence of experimental pneumococcal,[13] mycobacterial,[14] and aspergillus [15] infections.

In the present study, we undertook to define the natural history of cryptococcal meningitis in normal and cortisone-treated rabbits.

Materials and Methods

Organisms

Except when otherwise specified, we used a medium-capsule strain of *C neoformans* that was first isolated from the CSF of a 27-year-old male with Hodgkin's disease. This strain (DP) was identified in the Duke University Medical Center Mycology Laboratory by standard methods and shown to be subtype A by Dr. John E. Bennett. We also studied three other strains of *C neoformans.* These were: 1) strain BR, a recent isolate from a patient with recurrent chronic meningitis, 2) the original large-capsule strain that was first identified in 1894 by Busse and by Buschke [1] (from the personal collection of Dr. N. F. Conant), and 3) ATCC 68, a small-capsule strain, also subtype A (Dr. John Bennett's "No. 1" strain).

Method of Inoculation

Organisms were transferred from Sabouraud's agar slants at room temperature onto Columbia blood agar base containing 100 μg/ml of chloramphenicol, then incubated for 4–5 days at 35 C. Yeasts were taken up on cotton swabs and suspended in 0.015 M phosphate-buffered saline (PBS), pH 7.4, at an optical density of 1.5 ± 0.2 at 540 nm. This suspension contained $4.4 \pm 1.6 \times 10^7$ ($\pm$SE, n=8), colony-forming units (CFUs) per ml, determined by colony counting after spreading 0.1 ml from serial 10-fold dilutions in PBS onto agar and incubating at 35 C. New Zealand white male rabbits weighing 2–3 kg were housed in separate cages and supplied with Purina Rabbit Chow and water. Males were used because females appear to be relatively resistant, at least among guinea pigs and humans. Each animal was sedated with fentanyl and droperidol (Innovar, McNeil Laboratories, Irvine, Calif) 0.3 ml/kg intramuscularly. Ten minutes later, 0.3 ml of the suspension of cryptococci, containing approximately 1×10^7 CFU, was injected into the cisterna magna of each rabbit through a 25-gauge needle. Lower inocula containing 10^5, 10^3, and 10^2 CFU were prepared from the original suspension by making serial dilutions in PBS. The immediate mortality of intracisternal inoculation was less than 5%.

Administration of Cortisone

We injected a suspension of cortisone acetate, 50 mg/ml, supplied by Merck, Sharp & Dohme (West Point, Pennsylvania). Twenty-four hours before inoculating the rabbits with *C neoformans,* we began intramuscular treatment with cortisone, according to one of the following regimens: 0.25 mg/kg/day, 1.0 mg/kg/day, 2.5 mg/kg/day, 25 mg/kg/day, or 25 mg/kg/week. Each rabbit was given the same dose throughout, irrespective of any change in weight during the experiment.

Examination of CSF

CSF was aspirated at intervals for culture. To quantitate cryptococci, we inoculated agar plates with 0.1 ml of undiluted CSF and 0.1 ml of CSF diluted serially in PBS, incubated them for 72–96 hours at 35 C, and counted colonies. Swabs obtained from the brain surface of animals that died were streaked on agar. Leukocytes in CSF were counted in standard hemocytometers after appropriate dilution in Turk's solution. We determined glucose and protein concentrations using a du Pont ACA autoanalyzer.

Cryptococcal polysaccharide antigen levels in CSF were determined using the Crypto-LA Kit (International Biologic Laboratories, Rockville, Md). After thawing CSF that had been stored at -70 C and centrifuging it at 1500 rpm for 15 minutes, we made doubling dilutions of the supernatant in microtiter plates, using 0.025-ml microdiluters. An equal volume of sensitized latex particles was added to each well, and after 10 minutes of agitation on a shaking table the presence of agglutination was recorded with a microtiter plate reader. This technique was shown to give results identical to those obtained with the conventional method of performing the test on Boerner slides rotated 160 times per minute for 10 minutes. Our preliminary observations on more than a hundred samples of rabbit CSF or serum showed no false-positive reactions, nor was a rheumatoid factor detected in any of these samples. Thereafter, we did not heat inactive samples at 56 C for 30 minutes and did not include rheumatoid factor controls. We determined titers for all CSF specimens on the same day, using 5 μg and 0.05 μg of cryptococcal capsular polysaccharide as standards (Meridian Diagnostics Inc., P.O. Box 44216, Cincinnati, Ohio). Results were expressed as μg/ml of polysaccharide antigen.

Dissemination Studies

On Days 7 and 14 after inoculation, a rabbit from each cortisone treatment group was killed by intravenous injection of pentobarbitone. To determine whether dissemination had occurred, we removed sections of liver, lung, spleen, and kidney, weighed them, and homogenized them in 2 ml of PBS using glass tissue-grinders. We plated 0.2 ml of tissue homogenate to determine the number of CFUs per gram of tissue. We incubated 7–10 ml samples of heart blood, removed when the animals were killed in Columbia (Difco) broth with sucrose for 4–5 days, then plated them on Columbia agar with chloramphenicol for isolation of cryptococci.

Histopathology

Brains were fixed in 10% formalin–PBS. Sections were subsequently cut at 5 μ and stained with hematoxylin and eosin, alcian blue, or methenamine silver.

Temperatures

We took rectal temperatures using a veterinary thermometer (Cornell-Weinhagen).

Studies with Other Strains

Using the techniques described, we also inoculated 10^7 CFUs of strain BR, the Busse-Buschke strain, and strain ATCC 68 into the cisternal fluid of rabbits treated with cortisone 2.5 mg/kg/day. Quantitative CSF counts were made at 4, 7, 14, 21, and 28, days.

Statistics

Significance levels between groups were determined by applying the *t* test for unpaired means or chi-square analysis.

Results

Normal rabbits injected intracisternally with *C neoformans* showed no overt signs of disease. Cortisone-treated animals inoculated with cryptococci developed chronic progressive illness characterized by weight loss, lethargy, and low-grade fever, sometimes accompanied by stiffness of the neck with hyperextension or neurologic abnormalities such as nystagmus, ataxia, jerky head movements, paresis, and incontinence.

The mortality among infected and uninfected animals receiving cortisone is shown in Table 1. Most infected rabbits not receiving cortisone looked well throughout a 6-week period of observation. Mortality among infected rabbits increased to 100% as the daily dose of cortisone was increased. The length of survival of infected rabbits ranged from 11 to 80 days and was inversely related to cortisone dose. Thus, we observed that cortisone treatment increased mortality and shortened survival time in a dose-related fashion among infected rabbits. The low mortality among 18 rabbits receiving no steroid indicates that deaths were not due to the procedure of repeated CSF aspirations.

Treatment with high-dose cortisone alone (without inoculation of

Table 1—Mortality in Infected and Uninfected Animals That Received Varying Doses of Cortisone

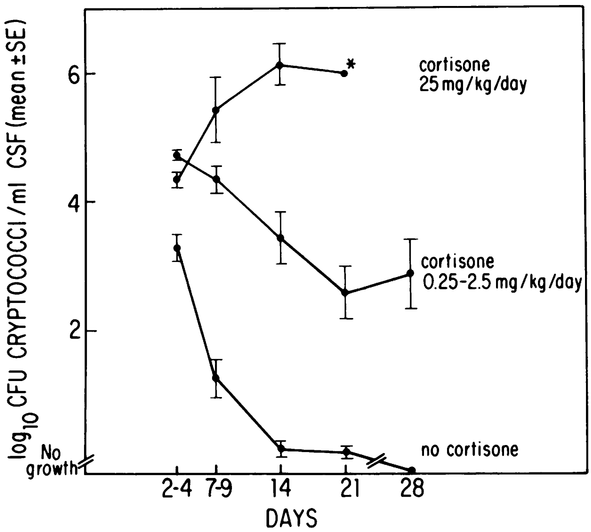
Cortisone dose (mg/kg/day)	Infected		Uninfected		P value (infected vs uninfected)
	deaths/total	%	deaths/total	%	
None	1/18	6*	NT†		—
0.25	4/9	44	NT		—
1.0	9/10	90	0/7	0	$P < 0.001$
2.5	15/16	94	5/20	25	$P < 0.001$
25	16/16	100	7/7	100	NS

* One rabbit only; postmortem brain swab was sterile.
† Not tested.

cryptococci or repeated CSF aspiration) caused wasting and resulted in high mortality. Seven rabbits receiving the highest dose (25 mg/kg/day) lived approximately 1 week longer than infected animals given this dose of cortisone, but all wasted and died within 4 weeks, often after developing severe diarrhea. Five of 20 rabbits given 2.5 mg/kg/day died before the experiment was terminated at 6 weeks, while at the lowest dose tested (1.0 mg/kg/day for 6 weeks) there were no deaths among 7 uninfected rabbits.

At necropsy 1–7 weeks after inoculation the brains of infected, cortisone-treated rabbits showed basilar meningitis. We found a gray mucoid exudate, most marked on the ventral surfaces, but sometimes extending upward over the cerebellar hemispheres. On microscopic examination 1–2 weeks after inoculation, the meninges investing the brain stem were found to be severely affected (Figure 1A). There was a dramatic contrast between the histologic appearance of the meninges in infected rabbits not given cortisone and in those treated with 25 mg/kg/day. In rabbits not receiving cortisone, the meninges were grossly thickened by an exuberant mononuclear cellular reaction. The subarachnoid space was filled with mononuclear cells, and only a few scattered cryptococci could be identified in alcian-blue–stained sections (Figure 1C). In contrast, cortisone-treated rabbits showed very few mononuclear cells, but the subarachnoid space was packed with masses of encapsulated yeasts (Figure 1D). Many yeast cells were free in the subarachnoid space, while many others had been ingested by mononuclear phagocytes (Figure 1B). Although parenchymal involvement of the brain was uncommon, occasional focal collections of cryptococci, devoid of host-derived inflammatory cells, were found in the cortex (Figure 1A). All the pathologic findings in cortisone-treated rabbits closely resembled those observed in fatal human cases.[1]

Quantitative cultures of CSF at intervals from 4 to 28 days after inoculation of approximately 10^7 CFUs of *C neoformans* demonstrated that normal rabbits eradicated cryptococci from this site (Text-figure 1). Four days after inoculation, rabbits not receiving cortisone all had positive CSF cultures, but the number of CFUs/ml was already significantly lower than in cortisone-treated animals (Text figure 1, $P < 0.001$). CSF was sterile in 16 of 18 rabbits not receiving cortisone 2 weeks after infection and in all 6 animals that were studied at 4 weeks. In contrast, rabbits treated with daily cortisone showed persistence of organisms in the CSF throughout the 4-week period of this study, with colony counts ranging from 10^1 to 10^6 CFUs/ml. Four days after inoculation the numbers of cryptococci in CSF were similar in all 4 cortisone-treated groups, with little variation within each group (Text-figure 1). The number of organisms in CSF of the



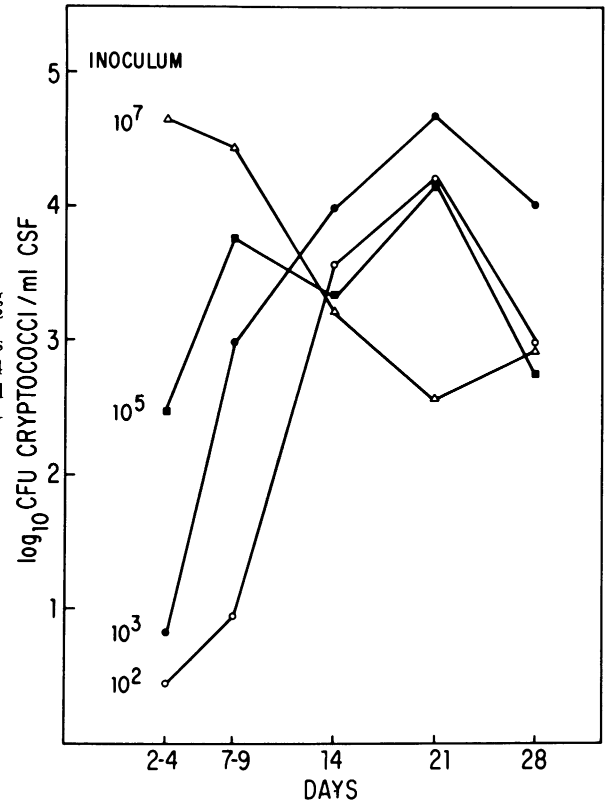
TEXT-FIGURE 1—Counts of viable *C neoformans* from CSF of 86 cortisone-treated and infected control rabbits over 4 weeks after inoculation of 10^7 CFUs. *SE not shown, because only two rabbits in this group survived for 3 weeks.

rabbits receiving cortisone 25 mg/kg/day increased progressively until death, while most animals receiving smaller doses showed a moderate reduction in counts of yeast between 1 and 4 weeks but remained culture-positive (Text-figure 1). Quantitative CSF cultures were not attempted on rabbits that had died, but postmortem brain swabs were positive in 43 of 45 infected, cortisone-treated animals.

The effect of varying inoculum size between 10^2 and 10^7 CFUs in rabbits treated with cortisone 2.5 mg/kg/day is shown in Text-figure 2. At 4 and 7 days, fewer organisms were found in CSF of rabbits receiving smaller inocula. However, by 14, 21, and 28 days the number of yeasts in CSF of all groups was similar. Thus, inoculation of as few as 100 CFUs of *C neoformans* was sufficient to cause chronic meningitis. Varying the inoculum size by as much as 10^5 CFUs did not significantly alter the long-term outcome.

Mononuclear cells greatly outnumbered polymorphonuclear leukocytes in the CSF of both steroid-treated and untreated animals (Table 2). Infected rabbits that received no cortisone responded with a brisk mononuclear reaction. The number of cells peaked 7–9 days after inoculation of cryptococci in all groups. Cortisone treatment caused a striking dose-related reduction in the mononuclear cell count in CSF (Text-figure 3). The smaller population of polymorphs was also reduced by cortisone in a dose-related fashion.

Cryptococci were found outside the CNS in all treatment groups (Table 3). Five of 9 blood cultures drawn 7 and 14 days after inoculation of cryptococci into rabbits receiving cortisone 1.0–25 mg/kg/day were posi-

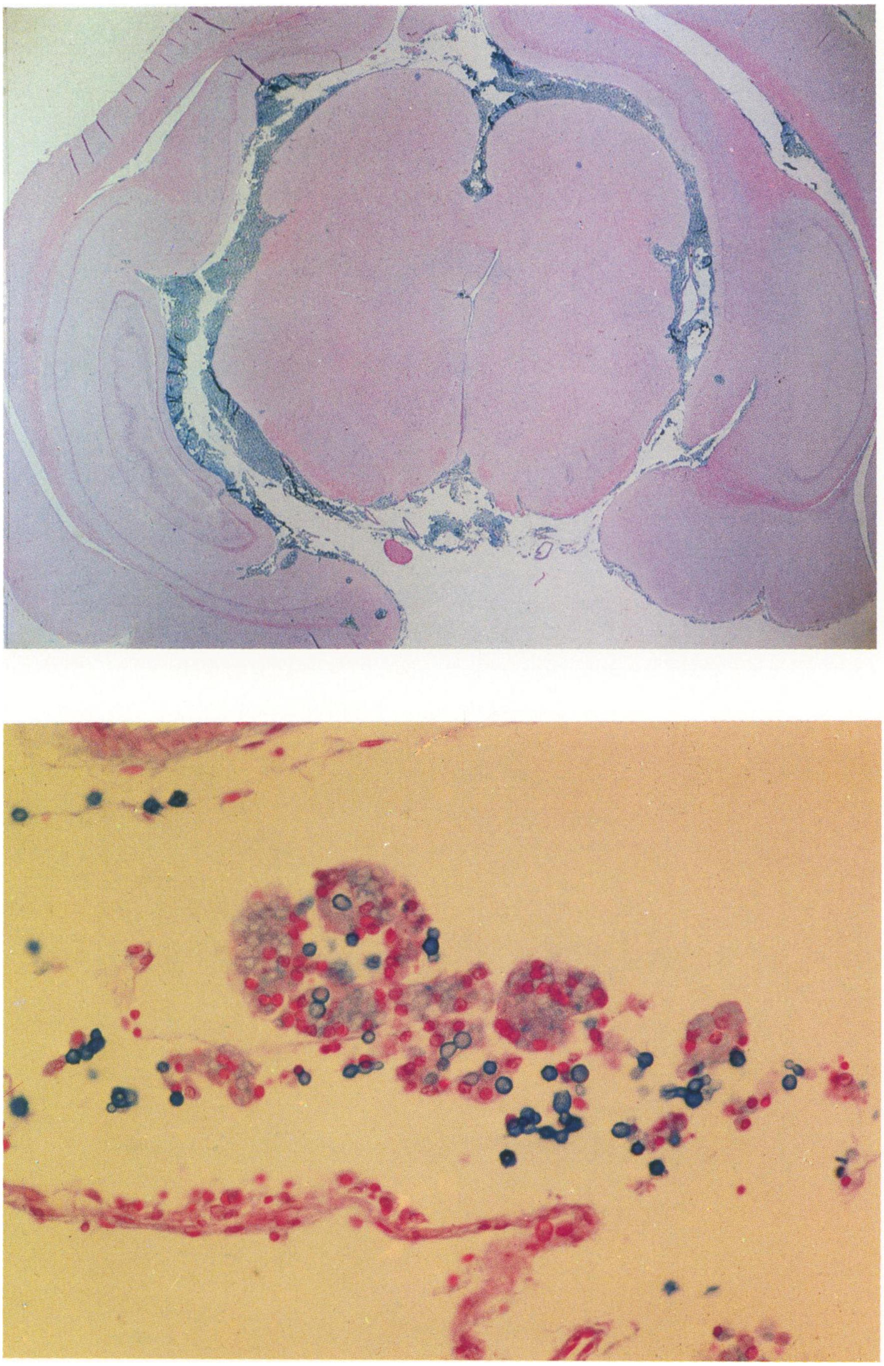


TEXT-FIGURE 2—Effects of varying the inoculum of strain DP from 10^2 to 10^7 CFUs on quantitative counts in CSF drawn at intervals over 4 weeks from 46 rabbits that received cortisone 2.5 mg/kg/day intramuscularly.

tive. The lungs consistently contained the highest number of organisms per gram of tissue among the organs cultured. Although the number of crypotococci in individual organs was variable, and less predictable than in CSF, culture-proven dissemination persisted for at least 28 days in cortisone-treated rabbits. In rabbits not treated with cortisone, cryptococci had been eradicated from all sites cultured by 14 days.

CSF protein and glucose levels were determined in specimens collected at intervals over the 14 days following inoculation. Rabbits treated with 2.5 mg/kg/day of cortisone had higher plasma glucose levels than those not receiving cortisone (mean 247 ± 30 mg/100 ml vs 167 ± 12 mg/100 ml). In all rabbits with positive CSF cultures the mean ratio of CSF to plasma glucose was lower than that in uninfected animals (0.47 ± 0.06 vs 0.6 ± 0.1), but this difference was not statistically significant. Infected rabbits not treated with cortisone had the lowest CSF glucose levels. The CSF protein levels in infected rabbits were raised during the first week (mean 127 ± 35 mg/100 ml) but approached normal by 14 days (mean 46 ± 6 mg/100 ml vs 39 ± 12 mg/100 ml).

Figure 1—Sections of brain and meninges from rabbits with cryptococcal meningitis, 1-2 weeks after inoculation. **A**—A cross-section in the region of the pons, showing widespread meningeal infiltration with blue-staining yeasts. Note occasional cystoid cryptococcal lesions in the parenchyma. (Alcian blue, ×5). **B**—Cells in the subarachnoid space. Some yeasts are free in CSF, while others have been ingested by mononuclear phagocytes. (Alcian blue, ×400) **C**—The subarachnoid space of an infected rabbit not treated with cortisone, showing abundant mononuclear cells but very few cryptococci. (H&E, ×250) **D**—Contrasting appearance in an infected, cortisone-treated animal, in which the subarachnoid space is packed with a multitude of yeasts, but very few inflammatory cells are present. (Alcian blue, ×100)



A

1

B

C

1

D

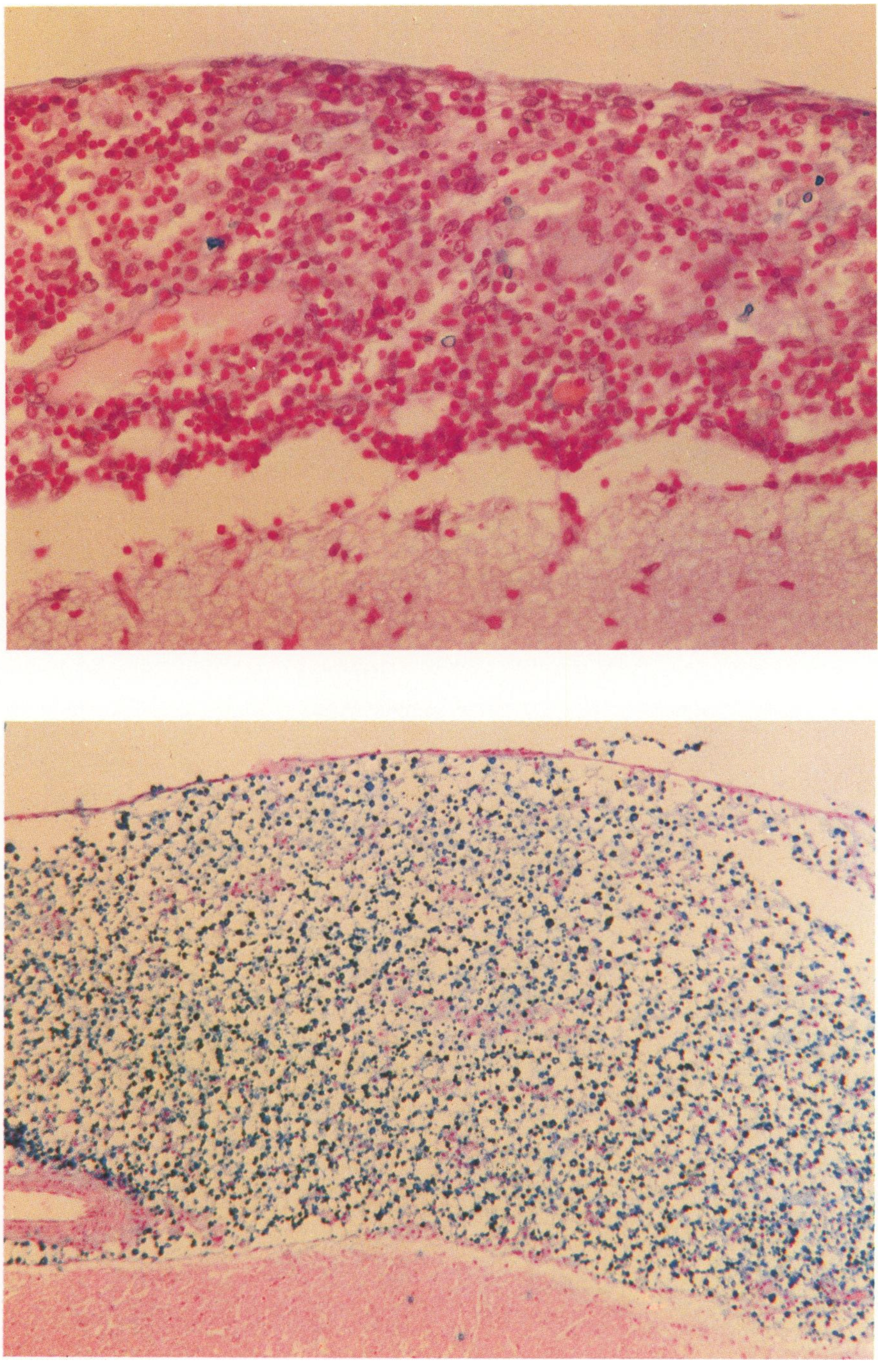
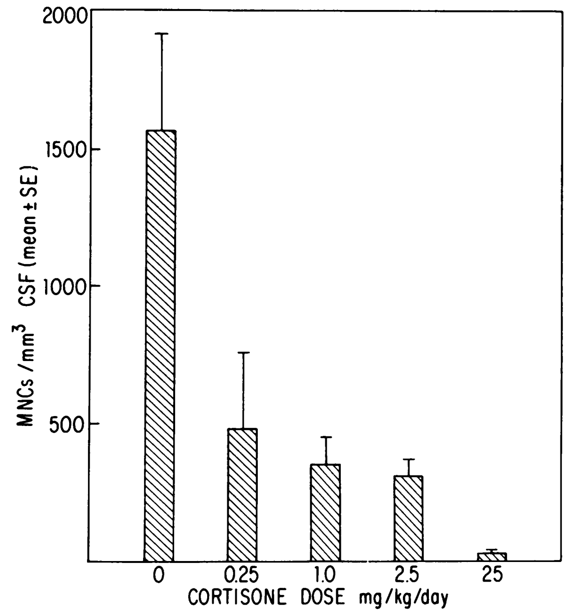


Table 2—Number of Polymorphonuclear (PMN) and Mononuclear (MNC) Leukocytes in CSF of Surviving Rabbits with Cryptococcal Meningitis

Duration of infection	Mean cells per cu mm ± SE (number)					
	No cortisone		Cortisone 0.25–2.5 mg/kg/day		Cortisone 25 mg/kg/day	
	PMN	MNC	PMN	MNC	PMN	MNC
Days 2–4	67 ± 27	965 ± 339 (21)	11 ± 5	167 ± 26 (41)	6 ± 3	24 ± 10 (21)
Days 7–9	149 ± 84	1567 ± 353 (17)	25 ± 6	305 ± 48 (29)	0 ± 0	26 ± 10 (8)
Days 14–16	15 ± 6	248 ± 51 (15)	6 ± 3	138 ± 27 (19)	1 ± 1	9 ± 5 (4)
Days 21–22	1 ± 1	62 ± 19 (8)	3 ± 2	116 ± 25 (14)	no survivors	
Days 28–30	0 ± 0	29 ± 7 (6)	11 ± 5	101 ± 34 (12)	no survivors	



TEXT-FIGURE 3—Number of mononuclear cells in CSF of rabbits on varying doses of cortisone 7–9 days after inoculation of 10^7 CFU cryptococci.

Four days after inoculation of *C neoformans* into CSF, all 26 rabbits not treated with cortisone and all 16 animals receiving cortisone 2.5 mg/kg/day had CSF polysaccharide antigen levels of $\geq$ 0.32 μg/ml. Seven days after inoculation, the CSF of all 20 cortisone-treated animals and 12 of 14 rabbits not treated with cortisone contained $\geq$ 0.32 μg/ml of polysaccharide antigen (range 0.32–5.4 μg/ml). Thus, at 1 week a substantial level of polysaccharide antigen persisted in the CSF of most rabbits, even though there were already significantly fewer viable organisms in the CSF of those not receiving cortisone (Figure 2, $P < 0.001$). By 14 and 28 days polysaccharide antigen was undetectable in the CSF of 10 normal rabbits, while 7 of 9 cortisone-treated rabbits still had levels of $\geq$ 0.08 μg/ml ($P < 0.01$ by chi-square analysis).

Table 4 shows the mean rectal temperature of infected and uninfected rabbits, receiving or not receiving cortisone. Treatment with cortisone did not cause hypothermia. In fact, experimental cryptococcosis in rabbits was a mildly febrile disease in its early stages.

Cortisone was administered daily in all the experiments described above. Because less frequent injections would be more convenient, we tested the effect of treating rabbits with cortisone acetate 25 mg/kg/day intramuscularly given 1 day before inoculation and once weekly thereafter. On this regimen, we found persistently high yeast counts at 4, 7, and

Table 3—Quantitative Yeast Counts per Gram of Tissue in 13 Rabbits that Received Varying Doses of Steroid, 7 and 14 Days After Inoculation of Cryptococci

Duration of infection	No cortisone	Log_{10} CFU/gm tissue Cortisone (mg/kg/day) 0.25	1.0	2.5	25
7 days					
Lung	2.6	3.3	3.5*	4.8*	4.7
Spleen	1.7	2.3	2.8	3.6	4.6
Kidney	0.0	1.6	1.2	3.4	3.3
Liver	0.0	1.6	1.4	2.5	2.6
14 days					
Lung	0.0	1.7	6.0	2.7*	4.2
Spleen	0.0	1.8	5.7	2.5	3.5
Kidney	0.0	0.0	5.0	2.4	0.0
Liver	0.0	1.6	4.9	1.8	0.0

* Mean of 2 rabbits.

14 days ($\geq 10^{4.5}$ CFUs/ml CSF), and 4 of 5 rabbits had died by 21 days. Thus intermittent cortisone injections at weekly intervals are adequate to predispose rabbits to progressive cryptococcal infection.

To determine whether meningitis could be produced by other routes of inoculation, we injected 10^7 CFUs cryptococci directly into the lung parenchyma of 5 rabbits and intravenously into 2 rabbits (treated with cortisone 1–25 mg/kg/day). Cryptococcal meningitis did not develop in any of these animals.

In all experiments described to this point we had used the DP strain of *C neoformans*. When we inoculated 10^7 CFUs of three other strains into rabbits treated with cortisone 2.5 mg/kg/day, the response was less predictable. Although the BR strain was cultured regularly from CSF over a period of 3 weeks, 2 of 5 rabbits inoculated with this strain survived, clearing the organisms from CSF by 4 weeks despite continuing administration of cortisone. All 6 rabbits given the Busse-Buschke strain died within 6 weeks, but in these animals recovery of the organisms from CSF was inconsistent. Recovery of cryptococci from CSF of 4 animals inoculated intracisternally with Bennett's "No. 1" strain (ATCC 68) was also inconsistent. Thus different strains showed different degrees of virulence, even in immunosuppressed rabbits. Further studies will be necessary to determine the true extent of these variations in the virulence of individual strains and the reasons for it.

Discussion

We have defined the salient features of chronic cryptococcal meningitis in rabbits, a species hitherto regarded as highly resistant to infection with

Table 4—Rectal Temperatures of Infected and Uninfected Rabbits That Did or Did Not Receive Cortisone

Group of rabbits	Duration of infection	No. of animals	Temperature (C) (mean ± SE)
Normal	—	8	39.0 ± 0.2
Infection only	1 week	10	39.9 ± 0.7
Cortisone only	—	8	39.4 ± 0.5
Cortisone + infection	1 week	13	40.1 ± 0.7
Cortisone + infection	4–5 weeks	8	39.1 ± 0.2

C neoformans. After intrathecal injection of yeasts, normal rabbits recovered promptly, but cortisone-treated animals developed chronic progressive infection, characterized by basilar meningitis with mononuclear pleocytosis, positive CSF cultures, and the presence of cryptococcal antigen. Most infected, cortisone-treated rabbits died within four weeks; time of death ranged from 11 to 80 days after inoculation. In established infection, the number of viable yeasts in CSF was directly related to the dose of cortisone rather than to inoculum size. In contrast, the number of mononuclear cells in CSF was inversely related to cortisone dosage.

Cortisone-treated rabbits provide a satisfactory animal model for both cryptococcosis and for chronic meningitis. Cryptococcosis occurs naturally in diverse animal species, causing central nervous system (CNS), pulmonary, or disseminated disease in cats, dogs, pigs, horses, cows, monkeys, and cheetahs.[16] Unfortunately, most of these animals are inconvenient for use in the laboratory. Mice are highly susceptible to experimental infection with *C neoformans*, but because of their small size, repeated aspiration of useful volumes of CSF is difficult or impossible. We have now shown that cortisone-treated rabbits inoculated intracisternally with *C neoformans* consistently develop chronic meningitis, which can be followed easily by repeated aspiration of CSF in volumes adequate for most tests.

Cryptococcosis in cortisone-treated rabbits has striking similarities to the disease in man. Like normal human beings,[17] normal rabbits possess strong natural resistance to progressive infection with *C neoformans*. Although human beings are regarded as steroid-resistant in comparison with rabbits,[18] corticosteroid therapy enhances infection in both species.[19,20] Several groups of investigators have drawn attention to subtle alterations in immune function that are frequently associated with progressive cryptococcal disease in humans.[1,4,5,21] Whether the immune defects induced by corticosteroid treatment of rabbits are qualitatively similar to those found in man remains to be investigated, but clinical and pathologic parallels are already apparent. In both species a chronic basilar meningitis occurs, associated with a mononuclear pleocytosis in CSF. Infected rabbits share many of the adverse prognostic factors defined in human disease by Diamond and Bennett [22]: corticosteroid therapy, low CSF leukocyte counts, presence of cryptococci on direct examination of CSF, high titers of cryptococcal antigen in CSF, and dissemination to other organs.

Although this paper is primarily concerned with the natural history of experimental cryptococcosis, our observations offer some preliminary insights into pathogenesis. The crucial role of corticosteroids in predisposing these animals to chronic infection with *C neoformans* is clearly illustrated by the finding that inoculum size had little influence on the ability of this organism to produce chronic meningitis. The effect of corticosteroids on cryptococcal infection in rabbits may be related to the dose-dependent reduction in CSF mononuclear cell response, which we have observed both in aspirated CSF and on histologic examination of the meninges. Sections from rabbits with progressive infection showed that mononuclear phagocytes had ingested large numbers of yeasts (Figure 1). These observations raise the hypothesis that the inability to resist infection was due to failure of a lymphocyte-mediated response to challenge with cryptococci. The rabbit model clearly disproves the long-favored theory that natural immunity to cryptococcosis in this species is due to high body temperature. In fact, experimental cryptococcosis in rabbits is a mildly febrile disease.

The advent of this simple animal model for chronic meningitis is timely in view of increasing interest in the immunobiology of the CNS.[23-26] The concentration of immunoglobulins and complement found in CSF is low, and the CNS is devoid of lymphoid tissue; it therefore probably represents an immunologically deprived compartment.[23] This concept could partially explain the predilection of certain low-grade pathogens such as *C neoformans* to localize and cause disease in the CNS. A crucial advantage of the rabbit model is that the immune response of highly resistant, normal rabbits can be compared and contrasted at every stage with the responses of highly susceptible, cortisone-treated rabbits, thus facilitating investigations of pathogenesis.

This model should also find application in treatment studies. Although a plethora of safe and effective antibacterial agents are available to the clinician, few antifungal drugs are presently available, and even these few are relatively toxic. In particular, there is urgent need for agents capable of eradicating fungi from the CNS. A simple, practical model is needed to adequately evaluate candidate drugs and new combinations of exsisting antifungal agents. Since drugs can be administered orally, intravenously, intramuscularly, or intracisternally to cortisone-treated rabbits, this model should prove useful for testing antifungal agents prior to clinical trials.

Thus, cortisone-treated rabbits provide a new and expedient laboratory model for cryptococcal disease. Potential applications include study of the pathogenesis of cryptococcosis, investigation of the immunobiology of the CNS in chronic meningitis, and *in vivo* evaluation of newer antifungal regimens.

References

1. Littman ML, Zimmerman LE: Cryptococcosis. New York, Grune & Stratton, 1956, pp 71–82, 111–116
2. Lewis JL, Rabinovich S: The wide spectrum of cryptococcal infection. Am J Med 1972, 53:315–322
3. Spickard A: Diagnosis and treatment of cryptococcal disease. South Med J 1973, 66:26–31
4. Diamond RD, Bennett JE: Disseminated cryptococcosis in man: Decreased lymphocyte transformation in response to *Cryptococcus neoformans*. J Infect Dis 1973, 127:694–697
5. Graybill JR, Alford RH: Cell-mediated immunity in cryptococcosis. Cell Immun 1974, 14:12–21
6. Weiss C, Perry IH, Shevky MC: Infection of the human eye with *Cryptoccocus neoformans (Torula histolytica; Cryptococcus hominis)*: A Clinical and experimental study with a new diagnostic method. Arch Ophthamol 1948, 39:739–751
7. Strippoli V, Simonetti N, Cassone A: Effect of a tetracycline antibiotic on the experimental pathogenicity of *Cryptococcus neoformans*. Chemotherapy 1978, 24:290–296
8. Felton FG, Maldonado WE, Muchmore HG, Rhoades ER: Experimental cryptococcal infection in rabbits. Am Rev Resp Dis 1966, 94:589–594
9. Kass EH, Finland M: Adrenocortical hormones in infection and immunity. Ann Rev Microbiol. 7:361–388, 1953.
10. Louria DB, Fallon N, Browne HG: The influence of cortisone on experimental fungus infections in mice. J Clin Invest 1960, 39:1435–1449
11. Gadebusch HH, Gikas PW: The effect of cortisone upon experimental pulmonary cryptococcosis. Am Rev Respir Dis 1965, 92:64–74
12. Diamond RD: Effects of stimulation and suppression of cell-mediated immunity on experimental cryptococcosis. Infect Immun 1977, 17:187–194
13. Robinson HJ, Smith AL: The effect of adrenal cortical hormones on experimental infection. Ann NY Acad Sci 56:757–764
14. Lurie MB, Zappasodi P, Dannenberg AM, Cadona-Lynch E: The effect of cortisone and ACTH on the pathogenesis of tuberculosis. Ann NY Acad Sci 1953, 56:779–792
15. Lehmann PF, Reiss E: Invasive aspergillosis: Antiserum for circulating antigen produced after immunization with serum from infected rabbits. Infect Immun 1978, 20:570–572
16. Barron CN: Cryptococcosis in animals. J Am Vet Med Assoc 1955, 127:125–132
17. Atkinson AJ, Bennett JE: Experience with a new skin test antigen prepared from *cryptococcus neoformans*. Am Rev Respir Dis 1968, 97:637–643
18. Claman HN: Corticosteroids and lymphoid cells. N Engl J Med 1972, 287:388–397
19. Wilson TS, Fleming WA, Robinson FLJ, Nicholl B: Cryptococcal meningitis associated with steroid therapy. J Clin Pathol 1970, 23:657–663
20. Schroter GPJ, Temple DR, Husberg BS, Weil R, Starzl TE: Cryptococcosis after renal transplant: Report of ten cases. Surgery 1976, 79:268–277

21. Hoeprich PD: Infectious Diseases: A Modern Treatise of Infectious Processes. New York, Harper & Row 1977, p 902
22. Diamond RD, Bennett JE: Prognostic factors in cryptococcal meningitis. Ann Int Med 1974, 80:176–181
23. Moser RP, Robinson JA, Prostko ER: Lymphocyte subpopulations in human cerebrospinal fluid. Neurology 1976, 26:726–728
24. Link H: The immune response in human demyelinating diseases. Adv Exp Med Biol 1978, 100:529–543
25. Couch JR, Abdou NI, Sagawa A: Histoplasma meningitis with hyperactive suppressor T-cells in cerebrospinal fluid. Neurology 1978, 28:119–123
26. Plouffe JF, Silva J, Fekety R, Baird I: Cerebrospinal fluid lymphocyte transformations in meningitis. Arch Int Med 1979, 139:191–194

[*End of Article*]